International Journal of Systematic and Evolutionary Microbiology (2003), **53**, 527–532 DOI 10.1099/ijs.0.02227-0

Note

Psychromonas profunda sp. nov., a psychropiezophilic bacterium from deep Atlantic sediments

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A psychropiezophilic bacterium, strain 2825^T (=LMG 21260^T =JCM 11437^T), isolated from deep Atlantic sediments at a depth of 2770 m and a temperature of 2°C, was found by polyphasic analysis to represent a novel species of the genus *Psychromonas*, *Psychromonas profunda* sp. nov. It is a strict psychrophile and a moderate piezophile, whose degree of piezophily is increased markedly when the temperature is raised to 10°C. The piezophily of *P. profunda* is intermediate between that of the type species, *Psychromonas antarctica*, which is not piezophilic, and that of *Psychromonas kaikoae*, which is an obligate piezophile.

The deep oceanic piezosphere usually remains at a temperature of 1·5–3 °C at all latitudes. It is therefore an environment par excellence for the recovery of strict psychrophiles (highest growth temperature below 20 °C; Morita, 1975) that differ in their degree of piezophily or piezotolerance (the prefix 'piezo-' denotes pressure; see Yayanos, 1995). Up to now, all cultivable psychropiezophiles have been found to be γ-proteobacteria of the genera *Colwellia*, *Moritella*, *Photobacterium* and *Shewanella* (Kato *et al.*, 2000a; Barlett, 2000; Xu *et al.*, 2003), none of which is confined to deep-sea environments, however. Abyssal archaea have also been reported (DeLong *et al.*, 1994), but not yet cultivated.

In this study, we characterize a novel species of the recently described genus *Psychromonas*, which also belongs to the γ-subclass of the *Proteobacteria*. The type species, *Psychromonas antarctica*, was isolated from a high-salinity pond on the McMurdo ice-shelf (Mountfort *et al.*, 1998). Another species, *Psychromonas kaikoae*, which is psychrophilic and obligately piezophilic, was retrieved from the Japan Trench (Nogi *et al.*, 2002). A third species, *Psychromonas marina*, which is psychrophilic but not piezophilic, has also been described (Kawasaki *et al.*, 2002).

Strain 2825^T was isolated from Atlantic sediments on board the ship *Meteor* during the cruise GEOTROPEX '83 in August 1983. The water depth at the sampling station (latitude 16°56·1′N, longitude 17°55·5′W) was 2770 m and the temperature was 2·7 °C (Rüger & Tan, 1992). Samples were taken by means of a box-grab sampler with surface dimensions of 50 × 50 cm. Subsamples were drawn with a sterile corer from near the centre of the sediment surface in order to obtain unwarmed samples. Sediment from the upper 2 cm layer was suspended in cold 75 % sterile sea water and spread onto chilled sea-water agar plates prepared with a medium containing 1·5 g peptone, 0·5 g yeast extract, 0·01 g $FePO_4.4H_2O$, 750 ml sea water and 250 ml distilled water. Sampling and isolation methods have been described in detail by Rüger & Tan (1992).

The reference strains used in this study, *P. antarctica* DSM 10704^T, *P. kaikoae* JT7304^T and *P. marina* JCM 10501^T, were grown as described by Nogi *et al.* (2002). High-pressure cultivation was performed in the DEEPBATH system at the Japan Marine Science and Technology Center (Kato *et al.*, 1995) as reported previously (Yanagibayashi *et al.*, 1999).

Growth or test media were Bacto Marine agar 2216 and Bacto Marine broth 2216 from Difco and the half-strength artificial sea-water, vitamin- and trace element-supplemented medium of Rüger (1988). Cardinal temperatures under high-pressure conditions were determined in Bacto Marine broth as described by Nogi & Kato (1999) by following total cell counts microscopically with a haematocytometer and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 2825^T is AJ416756.

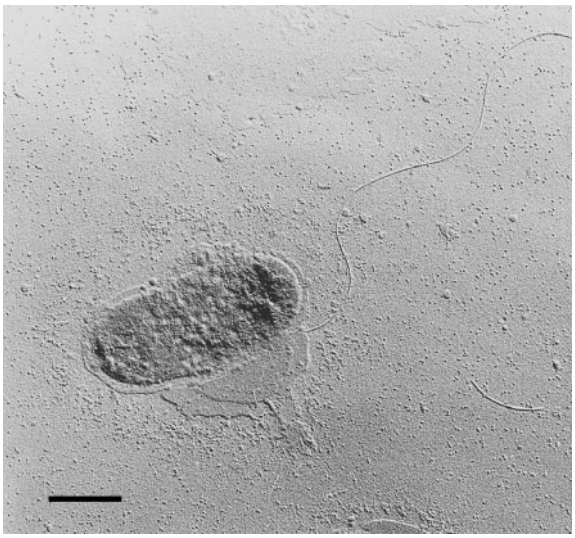

Fig. 1. Electron micrograph of a stained and shadow-cast cell of strain 2825^T. A drop of cell suspension (about 10^9 cells ml^{-1} in sterile water) was deposited on a filmed grid, fixed with osmium tetroxide and stained with 2% uranyl acetate. Shadowing was performed in a Balzers control unit (EVM, 052A, TCP27, 715 Hz) and the micrographs taken in a high-resolution electron microscope EM201C (Philips). Bar, 1 μm.

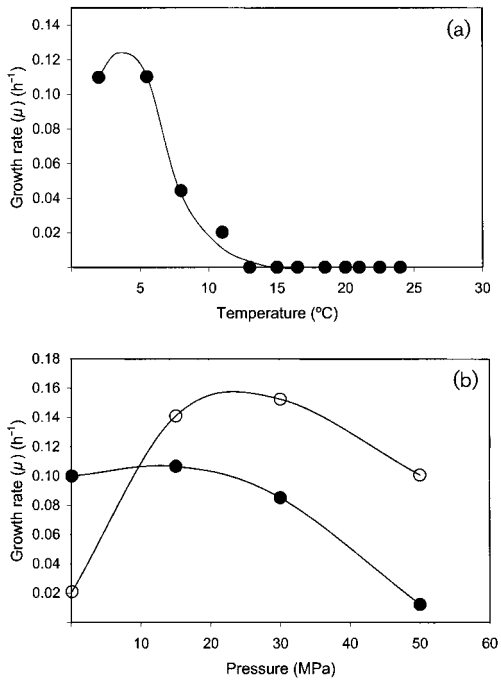

Fig. 2. (a) Effect of temperature on the growth rate of strain 2825^T under atmospheric pressure. (b) Growth rate of strain 2825^T under different pressures at 6 (●) and 10 (○) °C. The growth rate, μ, is calculated as $1/t_d$ [t_d is doubling time (h)].

culture samples fixed with formalin and stained with DAPI (4′,6-diamidino-2-phenylindole).

Cells of strain 2825^T are motile, Gram-negative rods. Under atmospheric pressure, cells are 0·9–1·2 μm wide and 2·0–5·5 μm long (Fig. 1). At the pressure where the maximal growth rate was observed (15–20 MPa at 6 °C; see Fig. 2), cells became slightly larger. At 50 MPa, which is about the limit at which cells still can grow at 6 °C, elongated forms were observed at low cell density.

Under atmospheric pressure, the maximal growth rate was obtained at a temperature of 3–4 °C (Fig. 2a). The strain grew in the range from 2 to 12–13 °C (no temperature below 2 °C was tested). On plates, very faint growth was observed after 14 days at 18 °C but there was no growth at all at 19 °C. Between 4 and 8 °C, growth yields remained approximately the same (about 10^9 cells ml^{-1}). No growth was observed in the absence of NaCl.

Strain 2825^T is moderately piezophilic. At 6 °C, the best growth was obtained at 15–20 MPa, less than that observed at the depth of isolation (2770 m). At 10 °C, however, the pressure for maximal growth rose to about 25 MPa (Fig. 2b).

Strain 2825^T was found to be facultatively anaerobic, oxidase-positive, chemo-organotrophic and prototrophic except for possible vitamin dependency (not tested). It produced acid from glucose and other carbohydrates (see Table 1 and the species description below) in the Minitek identification system (Becton Dickinson) (Rüger, 1981) and additionally, from glucose and lactose in Leifson's marine oxidation/fermentation medium (Leifson, 1963). It proved to be relatively oligotrophic: concentrations of 0·5 mg glucose, D-galactose or L-glutamate ml^{-1} already supported good growth on plates incubated at 4 °C on minimal medium supplemented with vitamins and trace elements (Rüger, 1988).

Thirty-four compounds were tested as carbon sources at a concentration of 1 g carbon l^{-1} in the above minimal medium at 4 °C and under atmospheric pressure; the results are reported in the species description. The strain proved sensitive to several antibiotics (as tested with Oxoid discs placed on sea-water agar plates; see description), including the vibriostatic agent O/129 (2,4-diamino-6,7-diisopropylpteridine phosphate).

The G+C content (Tamaoka & Komagata, 1984) of pure DNA (Saito & Miura, 1963) was 38·1 mol%, somewhat lower than observed for *P. antarctica*, *P. marina* and *P. kaikoae* (Table 1). The complete nucleotide sequence of the 16S rRNA gene was determined by direct sequencing of PCR-amplified DNA (Kato *et al.*, 1998). It presented 96·9 and 97·5% identity to sequences from *Psychromonas* sp. IC004 and *P. antarctica* DSM 10704^T, respectively. Identities were higher with *P. marina* JCM 10501^T (98·3%) and *P. kaikoae* (98·6%). On a distance phylogenetic tree constructed by the neighbour-joining method (Saitou & Nei, 1987) using the CLUSTAL W program (Thompson *et al.*,

Table 1. Phenotypic comparison of *Psychromonas* species

Characteristics are scored as: +, positive; −, negative; W, weakly positive after 3 weeks; (W), weakly positive after 6 weeks; NG, no growth. All strains are positive for catalase and oxidase and reduce nitrate to nitrite but not to gas; their major isoprenoid quinone type is Q-8; they can utilize D-galactose, maltose, mannitol and sucrose as sole carbon sources. All strains are negative for utilization as carbon sources and acid production from arabinose and raffinose; all are positive for utilization as a carbon source and acid production from maltose. No gas is produced from carbohydrates. *P. kaikoae* is distinguished by its obligate piezophily.

Characteristic	*P. antarctica* DSM 10704^T	*P. kaikoae* JCM 11054^T	*P. marina* JCM 10501^T	*P. profunda* JCM 11437^T
Maximum temperature for growth at 0·1 MPa (°C)	22	NG	25	14
Conditions for maximum growth rate:				
Temperature (°C)	12	10	15	3–4
Pressure (MPa)	0·1–10	50	0·1	30
DNA G+C content (mol%)	43·0	43·8	43·5	38·1
Gelatinase	+	+	−	−
Amylase	−	−	+	W
Indole production	−	−	−	+
Production of H_2S	−	−	+	+
Acid production from:				
Glycerol	+	W	+	+
Inositol	−	−	−	+
Lactose	−	−	+	+
Mannose	W	+	−	+
Rhamnose	−	−	−	+
Trehalose	+	+	−	+
Xylose	−	−	+	+
Utilization as carbon source:				
Glycerol	+	−	+	(W)
Cellobiose	−	+	+	+
Xylose	−	−	+	+

1994) without taking alignment gaps into consideration, the sequence was found to cluster with the 16S rRNA genes from the other *Psychromonas* species in a branch with high bootstrap support (Fig. 3). It is related to, but clearly distinct from, sequences from the genera *Moritella*, *Shewanella* and *Photobacterium*, which also comprise psychrophilic species found at different levels of the water column. In terms of similarity between sequences, the most closely related species were from the genera *Photobacterium* and *Vibrio*, with 90 % identity or less. The tree also contains *Moritella abyssi* and *Moritella profunda*, which are described in the accompanying paper (Xu *et al.*, 2003).

Reciprocal hybridizations were performed for 4 h at 35 °C between DNA extracted and purified (Saito & Miura, 1963) from strain 2825^T and *P. antarctica* DSM 10704^T, *P. marina* JCM 10501^T and *P. kaikoae* JCM 11054^T and monitored by fluorimetry (Ezaki *et al.*, 1989). Relatedness values all fell below 40 % (38, 38 and 24 % with *P. antarctica*, *P. marina* and *P. kaikoae*, respectively), while reciprocal values for the pairs *P. antarctica*/*P. marina*, *P. antarctica*/*P. kaikoae* and *P. marina*/*P. kaikoae* respectively averaged 48, 36 and 31 %. Since, by the current standard (Wayne *et al.*, 1987), distinct species of the same genus should be related at <70 % DNA–DNA relatedness, the data indicate that strain 2825^T represents a novel species of *Psychromonas*.

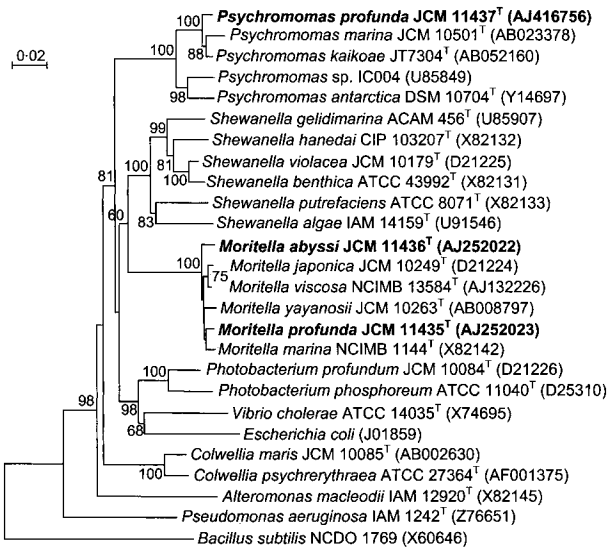

Fig. 3. Phylogenetic tree based on 16S rDNA sequences showing relationships between strain 2825^T (=JCM 11437^T) and other members of the *γ-Proteobacteria* using the neighbour-joining method. The bar represents 0·02 nucleotide substitutions per site. Bootstrap percentages were calculated from 1000 trees. GenBank accession numbers are given in parentheses.

Whole-cell fatty acids were analysed as described by Nogi *et al.* (1998) from cells grown in Bacto Marine broth. Major fatty acids in strain 2825^T were C14 : 1 (15 %), C16 : 0 (31 %) and C16 : 1 (44 %), a profile qualitatively similar to that of *P. marina*, which also displays small amounts of iso C16 : 0 and C22 : 6 (docosahexaenoic acid; DHA). *P. kaikoae* also contains C14 : 1 (10 %), C16 : 0 (15 %) and C16 : 1 (52 %) as major constituents, along with small amounts of both DHA and eicosapentaenoic acid, a distinctive feature of this species (Nogi *et al.*, 2002). In *P. antarctica*, the predominant fatty acids are C16 : 0 (24 %), C16 : 1 (58 %) and C14 : 1 (8 %) (Nogi *et al.*, 2002). The major isoprenoid quinone (Komagata & Suzuki, 1987; Nogi *et al.*, 1998) is Q-8.

The general phenotypic and biochemical profile of strain 2825^T is similar to those of *P. kaikoae*, *P. antarctica* and *P. marina* (Table 1). The main differences are the pressure response, strain 2825^T being intermediate between *P. kaikoae* and *P. antarctica*, and the more strictly psychrophilic profile of strain 2825^T.

The phenotypic and phylogenetic analysis of strain 2825^T thus shows that it belongs to the recently described genus *Psychromonas* (Mountfort *et al.*, 1998) and that it is sufficiently distant from the species already characterized to be considered as representing a novel species. We propose to call it *Psychromonas profunda* sp. nov. As with other members of the *Vibrionaceae*, it is facultatively anaerobic, capable of fermentative metabolism and is oxidase-positive. It is prototrophic except for possible vitamin dependency.

P. profunda is a strict psychrophile with a maximal growth rate at 3–4 °C under atmospheric pressure. As already noted for other psychropiezophiles (Yayanos, 1995; Abe *et al.*, 1999; Kato *et al.*, 2000b), growth of *P. profunda* at low temperature (6 °C) was enhanced at a pressure (15–20 MPa) lower than that found at the depth of isolation (28 MPa); moreover, incubating the cells at a higher temperature (10 °C) made them more piezophilic (the profile then peaked at about 25 MPa). This phenotype was also reported for thermopiezophiles retrieved from hydrothermal vents (Marteinsson *et al.*, 1997). Such behaviour could be explained by any biological process that is slowed down by an increase in pressure but favoured by an increase in molecular mobility. An increase in temperature could counteract the 'gelling' effect of high pressure on membrane lipids (Marteinsson *et al.*, 1997). It is also possible that pressure-induced compression critically affects the functioning of some enzymes in such a way that an increase in temperature can partially compensate for this effect.

Regarding the effects of pressure and temperature on proteins, psychropiezophiles are a living paradox since, on the one hand, efficient catalysis at low temperature requires high flexibility (at least in those parts of the molecules that are involved in the catalytic mechanism) and on the other hand, enzymes adapted to high pressure are expected to resist compression, thus to be more rigid (reviewed by Glansdorff & Xu, 2002). Some of these enzymes might be particularly sensitive to the opposite effects exerted by an increase in pressure and an increase in temperature. Furthermore, the difficulty in achieving such compromises may explain why abyssal psychropiezophiles grow relatively slowly. Careful nutritional studies will be required to test this proposal, but it is already noteworthy that 'with increasing pressure-adaptation in barophilic (piezophilic) isolates, the maximum growth rates at optimum pressures decrease' (Jannasch & Wirsen, 1984) (see also Yayanos *et al.*, 1982; Yayanos, 1995). Very few studies of enzymes from psychropiezophiles have been carried out (Bartlett, 2000). The availability of closely related psychrophilic organisms adapted to different sections of the water column is of considerable interest to molecular biologists, since it provides a paradigm to analyse the basis of functional adaptation of cold-active enzymes to the whole range of hydrostatic pressures found in the piezosphere.

Members of the genus *Psychromonas* have been found in places as distant as the Antarctic coastal area (*P. antarctica*), the deep Atlantic at a northern tropical latitude (*P. profunda*) and the Japan Trench (*P. kaikoae*). This suggests that the evolution of the genus *Psychromonas* has been influenced by deep-ocean water circulation (Nogi *et al.*, 2002). It would therefore be interesting to determine whether the same or only related species of *Psychromonas* also occur in the high Arctic. Until a short while ago, the general trend had been to observe the same genus but not the same species in the two polar domains (Staley & Gosink, 1999). In contrast, Rüger *et al.* (2000) reported that psychrophilic and psychrotolerant strains of the same species, *Bacillus marinus*, were indigenous to sediments of the Arctic and Antarctic oceans, the tropical Atlantic and the Iberian deep sea.

Description of *Psychromonas profunda* sp. nov.

Psychromonas profunda (pro.fun′da. L. fem. adj. *profunda* from the deep).

Cells are Gram-negative rods, either isolated or in pairs, 0·9–1·2 μm wide and 2·0–5·5 μm long, motile by means of a single, unsheathed and polar flagellum. On peptone/yeast extract/sea-water agar, colonies are smooth, colourless, translucent, irregular, punctiform and flat with an intact margin. Moderately halophilic (no growth observed in the absence of NaCl, normal growth with half-strength sea-water), strictly psychrophilic and moderately piezophilic. The temperature range for growth is 2 °C (or less, not tested) to 12–13 °C (14 °C on plates). Maximal growth is observed at 3–4 °C under atmospheric pressure. Growth is influenced favourably by pressure, with a maximum at 15–20 MPa at 6 °C and about 25 MPa at 10 °C. Facultatively anaerobic and prototrophic (with possible vitamin dependency, not tested) chemo-organotroph capable of both respiratory and fermentative metabolism. Catalase and cytochrome oxidase tests are positive. Nitrate is reduced to nitrite but no gas is produced. Indole and ONPG tests are positive. H_2S is produced from cysteine. Susceptible to discs (Oxoid) containing

150 μg O/129, 2 U penicillin G, 10 μg tetracycline, 10 μg chloramphenicol, 50 μg furazolidone and 300 U polymyxin B. The major isoprenoid quinone is Q-8. Predominant cellular fatty acids are C14 : 1, C16 : 0 and C16 : 1. Acid is formed oxidatively and fermentatively from glucose, lactose, cellobiose, dulcitol (weakly), fructose, galactose, glycerol, inositol, maltose, mannitol, mannose, rhamnose, salicin, sucrose, trehalose and xylose; no acid from adonitol, arabinose, melibiose, raffinose or sorbitol. Within 4 weeks at 4 °C, utilizes cellobiose, galactose, gluconate, maltose, salicin, sucrose (weakly), trehalose, xylose, fumarate, succinate, mannitol (weakly), citrate, pyruvate, L-alanine, L-aspartate, glutamate and putrescine as sole carbon and energy sources but not arabinose, α-D-glucose, mannose, ribose, propionate, adipate, β-hydroxybutyrate, sorbitol, L-arginine, L-histidine, L-ornithine, *p*-hydroxybenzoate or quinate. Acetate, DL-lactate and glycerol are utilized only after prolonged incubation for up to 6 weeks. Starch is hydrolysed only weakly and gelatin not at all. Aesculin is hydrolysed and the DNase test is positive. Lipase, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase tests are negative. The DNA G+C content of the type strain is 38·1 mol%.

The type and only strain, strain 2825^T (=LMG 21260^T=JCM 11437^T), was isolated from the upper layer of deep Atlantic sediments at a depth of 2770 m off the West African coast.

Acknowledgements

This work was supported by the Flanders Foundation for Joint Fundamental Research. We thank Mr Kurino and his co-workers for operating the DEEPBATH high-pressure microbial cultivation system and Mr J.-P. ten Have for the artwork.

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